Lab Session 10-06 to 22-06

08 August 2015 23:28

Lab Session 22.06.2015

Inoculation was set up for NrfA plasmid for further characterization.

- First day, inoculation was done in small scale.
- Next day, it was extrapolated to a larger scale.

Lab Session 17.06.2015

Gel Run

Sqr(digested)	8uL
Sqr(undigested)	3uL
Ladder	3uL
Loading Dye	2uL

Result:

Yet again, we failed to get satisfactory results.

Repeated failures in getting desired results forced us to think of alternatives or rather changes in our strategy.

Lab Session 16.06.2015

Once again, we set up digestion for Sqr:

Buffer 2.1	2 uL of 10 X
EcoRI	1 uL
PstI	1 uL
mQ	0 uL
Plasmid	4 uL
Total	8 uL

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Lab Session 15.06.2015

Gel run was done for the Sqr plasmids isolated.

Results:

The Sqr plasmid was cloned properly. However, resolution was not correct.

Lab Session 14.06.2015

Plasmid concentration has been regularly found to be low. So, there were some changes from the regular protocol for better results.

Changes from regular protocol:

- Pellet in micro-centrifuge tube for 3min at 15-25 degree rather than 10 min at 4 degree.
- Extended lysis (3-4 minutes).
- Wait for 2 minutes for ethanol evaporation before elution.
- Wait for 4-5 minutes after addition of elution buffer.
- One step elution (60 micro liters).

Readings (For Sqr plasmid, from 10 mL culture):

Conc	260/280	260/230
583.9 ng/uL	2.09	2.37

Inference:

- Clearly, a much higher concentration was obtained. However, there might be chances of contamination as well.

Lab Session 11.06.2015

We took nanodropic readings of the isolated plasmids, i.e CysI and Promoter.

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The results are:

	Concentration	260/280	260/230
Promoter	93.5	2.01	2.20
CysI	125.3	1.97	2.15

Inference:

- Concentration of both the samples were acceptable.

This done, we performed gel run of the digested Sqr.

Result:

However, no positive result was obtained. We obtained only a faint band of the Sqr gene

corresponding to 3.5 kB thereby suggesting that there was no digestion.

Sqr wasn't digested successfully even this time. Either the enzymes are not working

properly or the time for which digestion was done is not sufficient.

So, we planned to perform parallel digestions with different time periods for reassuring

Results before changing the enzymes.

Lab Session 10.06.2015

Digestion was done for: Sqr plasmid.

Buffer 2.1	2 uL of 10 X
EcoRI	1 uL
PstI	1 uL
mQ	0 uL
Plasmid	6 uL
Total	10 uL

Plasmid Isolation was performed for CysI and Promoter (J23119) using the kit

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(QIAprep Spin Miniprep Kit 50).

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